Antibotulinal Properties of Selected Aromatic and Aliphatic Aldehydes

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ABSTRACT

Aromatic and aliphatic aldehydes were tested for their effectiveness against *Clostridium botulinum* spores and cells. Six-tenths millimolar benzaldehyde, piperonal, phenylacetaldehyde, α-amylcinnamaldehyde, vanillin, or phenylglyoxal delayed germination in botulinal assay medium (BAM) broth after 6 h exposure at 32°C. Sporicidal activity was observed with 1.25 mM vanillin, 39 mM isobutyraldehyde, 156 mM pyruvaldehyde or valeraldehyde, 625 mM benzaldehyde, and 2,500 mM α-amylcinnamaldehyde. Twenty-five millimolars of cinnamaldehyde, phenylacetaldehyde, pyruvaldehyde, and vanillin were most active against vegetative cells at pH 7.0 in BAM broth, while 125 mM was required for benzaldehyde, acetaldehyde, piperonal, or phenylglyoxal. Three millimolars benzaldehyde, 5.0 mM phenylglyoxal, 150 mM cinnamaldehyde, 200 mM pyruvaldehyde and vanillin, and 300 mM piperonal inhibited 9 h dipicolinic acid release in BAM broth at 32°C. Spore resistance to a 20-min 80°C thermal treatment was reduced when challenged with prior exposure to 100 mM cinnamaldehyde, piperonal, pyruvaldehyde, vanillin, or phenylglyoxal. Inhibition by cinnamaldehyde, piperonal, and phenylglyoxal was retained in commercial canned chicken and in beef broths. Five millimolars of benzaldehyde, cinnamaldehyde, piperonal, pyruvaldehyde, or phenylglyoxal delayed neurotoxin production for 48 h at 32°C, while 25 mM was required for vanillin. These results indicate that certain aldehydes inhibit *C. botulinum*, and aromaticity improves efficacy.

Aldehydes are reactive carbonyl-containing compounds that have received widespread use as chemical intermediates (*21*). The activity of these compounds may be attributed to the polar nature of the carbonyl moiety and its susceptibility to oxidation and reduction reactions, nucleophilic additions, and the reactivity of the α-carbon. While a number of these compounds are approved as food additives by the Food and Drug Administration (FDA), current application is limited, and few have been thoroughly assessed for inhibition against foodborne pathogens (*2,4,12,16,20,23*).

A number of aldehydes have been shown to limit biological activity, or to alter critical growth parameters, such as nutrient availability. Of those aldehydes for which antimicrobial activity has been demonstrated, the mechanism of action is poorly defined, and knowledge of antigerminative activity against sporeforming bacteria is limited (*2,16*). Their limited application and inhibitory spectra necessitate ongoing research to identify new agents or novel approaches to retard microbial growth (*3*). The scope of the problem has been magnified moreover, by the emergence of a variety of "new generation" refrigerated foods that are minimally processed, convenient, and for which advance manufacturing and storage technologies are required. For example, the packaging conditions, minimal thermal treatments, and extended shelf lives of these products pose microbiological concerns about growth potential of bacterial pathogens such as *Clostridium botulinum.*

The hydrophobic character of bacterial spores (*7,31*), nutritional and germination requirements of sporeforming bacteria (*9,13*), and the chemical composition of spore coats afford opportunities to develop a variety of mechanistic approaches to inhibit these organisms. For example, the binding capacity of aldehydes to critical components needed for growth and survival, such as metal ions (*24,30*), sulfhydryl groups (*5,22*), amino acids, and proteins (*29*), suggests potential activity against endosporulating bacteria of public health concern. Few have been thoroughly assessed as growth inhibitors, however. Therefore, several aromatic and aliphatic aldehydes were tested in this study against *C. botulinum* to characterize their inhibitory potential and determine the relationship between structure and activity.

MATERIALS AND METHODS

Cultures

A spore mixture containing 3 type A (33, 62A, 69) and 3 type B (999, 169, ATCC 7949) proteolytic *C. botulinum* strains was used throughout the study. Individual strain spore suspensions were prepared by culturing in botulinal assay medium (*14*) without thioglycollate (BAM) for 21 d at 32°C in a flexible anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI). Anaerobiosis was maintained using a gas mixture consisting of 10% CO_2, 5% H_2, and 85% N_2, by periodic gas-exchange flushes and palladium catalyzed O_2 removal. Spore crops were harvested by three successive centrifugations at 17,310 × g for 10 min at 5°C with sterile distilled H_20 washes. Spore pellets were suspended in sterile distilled H_20, heat shocked (80°C/10 min), and stored at 5°C prior to use. *C. botulinum* confirmation was based on Gram reaction,

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cellular morphology, neurotoxin production by mouse bioassay, lipase, catalase, and oxidase activities (*6*). Each spore crop was quantified and the 6-strain spore mixture prepared by combining equal numbers of the individual strains to create a final concentration of 4.72×10^5 spores per ml. Viability and germination rates were tested initially on individual strains and monthly on spore mixtures (*6*). Spore suspensions were quantified prior to storage and immediately before antimicrobial testing to determine inoculum size.

Aromatic and aliphatic aldehydes

Benzaldehyde (99%), acetaldehyde (99%), cinnamaldehyde (99%), isobutyraldehyde (98%), piperonal (99%), phenylacetaldehyde (90%), α-amylcinnamaldehyde (97%), pyruvaldehyde (40%), vanillin (99%), phenylglyoxal (95%), heptaldehyde (95%), and valeraldehyde (98%) were purchased from Aldrich Chemical Company, Inc., Milwaukee, WI. Structures and some properties of the aldehydes are listed in Table 1. Stock solutions of the aldehyde compounds tested were prepared (wt/vol or vol/vol) in 95% reagent grade ethanol according to their respective normal states at 25°C and purities. Each aldehyde stock solution was stored at 5°C or at room temperature in an appropriate container, according to their respective reactivity. Final ethanol concentrations of treatments for antimicrobial testing were below those reported to be sporostatic and/or sporicidal (*17,19*).

Determination of spore minimal inhibitory concentrations (MIC)

A modified version of the Association of Official Analytical Chemists method of analysis for sporicidal activity of disinfectants was employed to determine the effect of aldehydes on *C. botulinum* spores (*1*). Test compounds were serially diluted (2500, 1250, 625, 312.5, 156.25, 78, 39, 19.5, 9.76, 4.88, 2.44, 1.22, 0.61, 0.30, 0.15 mM) in BAM broth and inoculated with 0.1 ml of a 4.72×10^5 CFU/ml heat-shocked (80°C/10 min) activated spore suspension. After anaerobic exposure for 6 h at 32°C, each test concentration culture was subcultured (0.1 ml) to five replicate tubes of thioglycollate broth prior to (sporicidal activity) and after heat-shock (80°C/10 min) treatment (sporostatic: antigerminative activity) to destroy germinated spores. Thioglycollate broth tubes were incubated aerobically for 48 h at 32°C, then examined for turbidity. Lack of growth in ≥4 thioglycollate broth tubes inoculated with non-heat- or heat-treated test cultures was interpreted, respectively, as sporicidal or sporostatic (i.e., antigerminative).

Dipicolinic acid (DPA) release

DPA release was estimated using the colorimetric assay of Janssen et al. (*15*). Test compounds were added to 9.9-ml BAM broth tubes to create a concentration series of 0.5, 1, 2, 3, 4, 5, 50, 100, 200, and 300 mM. Test media were inoculated with 0.1 ml of a heat-shocked (80°C/10 min) spore suspension (4.72×10^5 CFU/ml) and incubated 9 h anaerobically at 32°C. Nine-hour cultures were centrifuged at 1,500 × g for 10 min, and the supernatant fluid decanted for colorimetric analysis. One milliliter of a freshly prepared 0.5 M acetate-buffered chromogenic reagent was added to 4.0 ml of culture supernatant fluid. Optical density was measured at 440 nm using a Shimadzu UV-VIS Model 160 spectrophotometer (Kyoto, Japan) and DPA content calculated from a standard curve of known DPA concentrations (0-160 μg/ml).

Effect of aldehydes on spore thermal resistance

C. botulinum spores (8.20×10^6 CFU/ml) were aerobically exposed to 100 mM of each compound in 5.0-ml glass vials of BAM broth for 30 min at 25°C, and the exposure media transferred to a 80°C Exacal high temperature water bath (NesLab Instruments Inc., Newington, NH) for 5-20 min. Keithley Metrabyte datalogger Model DDL 4100 (Tauton, MA) was used to monitor temperature and equilibration times. Final heating temperature (80°C) was reached as follows: 83 s benzaldehyde, 85 s cinnamaldehyde and piperonal, 80 s pyruvaldehyde, 100 s vanillin, and 150 s phenylglyoxal. Samples were removed, cooled in an ice bath, plated in duplicate onto BAM agar plates using a Spiral Systems Model D plating instrument (Cincinnati, OH), and incubated anaerobically at 32°C for 48 h. Plates were enumerated using a Spiral Systems Model 500A, then converted into bacterial counts with Spiral Biotech CASBA™ II BEN software (Bethesda, MD). Spore thermal resistance was evaluated by comparing population densities of aldehyde-treated and untreated BAM samples. A 50-min exposure time control of nonheat-treated (25°C) spores with aldehydes was included to confirm spore viability in the absence of thermal treatment.

Inhibitory activity in chicken and beef broths

Commercially prepared canned chicken and beef broths were dispensed in 9.9-ml portions into sterile test tubes, and test compounds were added to yield 2.0, 3.0, 4.0, and 5.0 mM concentrations. The broths were inoculated with 0.1 ml of a heat-shocked (80°C/10 min) 4.72×10^5 CFU/ml spore suspension, incubated anaerobically at 25°C, and examined at 24-h intervals for turbidity. The commercial chicken and beef broths contained, respectively, 4.0 g protein, carbohydrate, and fat (chicken or beef); each at a final concentration of 1% (wt/vol).

Determination of vegetative cell MICs

The inhibitory effect of aromatic and aliphatic aldehydes on *C. botulinum* cells was tested at 4 concentrations (25, 125, 250, and 500 mM) and at 3 pH levels (6.0, 7.0, and 8.0) in BAM agar. Test compounds were added to BAM agar (100 ml) after autoclaving to yield the desired concentration range at each of three pH levels. The agar plates were surface streak inoculated with a 24-h culture of *C. botulinum* and incubated anaerobically for 48 h at 32°C. The growth response of the organism in the presence of aldehydes and the effect of pH on inhibitory activity were compared with those of unsupplemented BAM controls.

Effect of aldehydes on toxigenesis

Five-milliliter BAM broth tubes containing 5, 10, 25, 50, or 100 mM of the test compounds were inoculated with 0.1 ml of a heat-shocked (80°C/10 min) 4.72×10^5 CFU/ml spore suspension. Tubes were incubated anaerobically for 48 h at 32°C, then centrifuged (1500 × g /10 min) to remove cellular debris. A 72-h bioassay was conducted on duplicate Swiss-Webber 15- to 20-g mice of either sex by intraperitoneal injection of 0.5 ml undiluted culture supernatant fluid (*6*). Polyvalent antiserum (Center for Disease Control) controls were included on some samples to confirm the clinical symptoms as botulism.

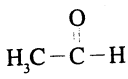
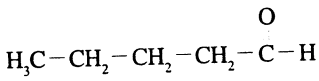
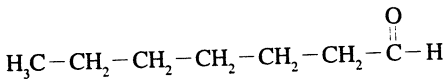
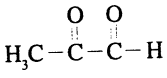
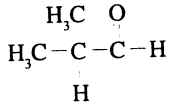
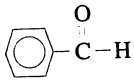
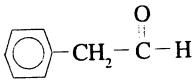
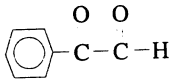
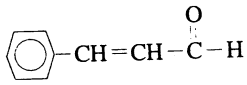
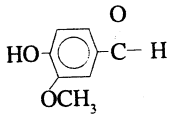
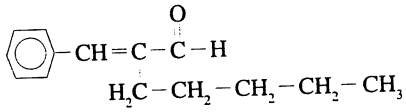
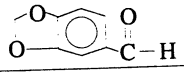
Structure/activity comparisons

The aldehydes tested were ranked according to their respective sporostatic, sporicidal, and vegetative cell MICs using Lotus 1,2,3 (Lotus Development Corporation, Cambridge, MA). Comparisons were based on chemical and physical properties, including: (i) carbon chain length; (ii) carbonyl number; (iii) boiling point; (iv) formula weight; and (v) the aromatic or aliphatic nature of the R-group.

RESULTS

Both aromatic and aliphatic aldehydes delayed germination of a 6-strain *C. botulinum* spore mixture after 6 h exposure at 32°C in BAM broth (Table 2). Benzaldehyde, piperonal, isobutyraldehyde, phenylglyoxal, α-amylcinnamaldehyde, vanillin, heptaldehyde, and phenylglyoxal exhibited sporostatic (i.e., antigerminative)

TABLE 1. *Aromatic and aliphatic aldehydes tested.*

Compounds	Structure	NS[a]	FW[b]	BP[c]
Acetaldehyde		L	44.05	21
Valeraldehyde		L	86.13	103
Heptaldehyde		L	114.16	153
Pyruvaldehyde		L	72.06	170
Isobutyraldehyde		L	72.11	63
Benzaldehyde		L	106.20	178 -179
Phenylacetaldehyde		L	120.15	195
Phenylglyoxal		S	134.10	142 /125mm
Cinnamaldehyde		L	132.16	250 -252
Vanillin		S	152.15	170 /15mm
α-amylcinnamaldehyde		L	202.30	287 -289
Piperonal		S	150.13	264

Structural and physical properties obtained from Aldrich Chemical Company, Inc., Milwaukee, WI.
[a] NS = normal state (liquid or solid) at 25°C.
[b] FW = formula weight.
[c] BP = boiling point.

activity at 0.6 mM. Pyruvaldehyde and valeraldehyde were antigerminative at 2.44 mM, while 9.78 mM acetaldehyde and cinnamaldehyde were required to prevent germination. Sporicidal (i.e., complete inactivation) activity was observed with 1.25 mM vanillin, 39 mM isobutyraldehyde, 156 mM pyruvaldehyde or valeraldehyde, 625 mM benzaldehyde, and 2500 mM α-amylcinnamaldehyde.

A select group of the most active inhibitors of *C. botulinum* spores was subjected to additional analyses to further define the antibotulinal properties of aldehydes; 5 aromatic (benzaldehyde, cinnamaldehyde, piperonal, vanillin, phenylglyoxal) aldehydes and 1 aliphatic (pyruvaldehyde) were tested. The parameters investigated included: (i) dipicolinic acid release, (ii) thermal resistance, (iii) antigerminative activity in commercial broths, and (iv) neurotoxigenesis. Benzaldehyde (0.5 mM) was the most active inhibitor of DPA release from *C. botulinum* spores after 9 h at 32°C (Fig. 1). DPA release was totally inhibited

with 3.0 mM benzaldehyde, 5.0 mM phenylglyoxal, 150 mM cinnamaldehyde, 200 mM of vanillin or pyruvaldehyde, and 300 mM piperonal.

The thermal resistance of proteolytic *C. botulinum* spores at 80°C was reduced when exposed for 30 min to 100 mM of cinnamaldehyde, piperonal, pyruvaldehyde, vanillin, or phenylglyoxal (Table 3). Phenylglyoxal was the most effective compound tested with a 3-$\log_{10}$ reduction in population density after 5 min at 80°C. A 4-$\log_{10}$ reduction at 80°C was observed after 20 min for spores exposed to piperonal and vanillin, at 15 min for cinnamaldehyde and pyruvaldehyde, and 10 min for phenylglyoxal. Benzaldehyde was least active in that only a 2-$\log_{10}$ drop in population density occurred after 20 min. Vanillin and phenylglyoxal were unique among the compounds tested in that 1- and 3-$\log_{10}$ reductions, respectively, in population density occurred during temperature equilibration to 80°C. Come-up times for treatments containing vanillin (100 s) and phenylglyoxal (150 s) were significantly greater than those of the other compounds tested (80-85 s). Population densities of nonheat-treated controls containing 100 mM aromatic or aliphatic aldehydes remained constant for the duration (20 min) of the test.

TABLE 2. *Inhibitory activity of aldehydes against* C. botulinum *spores at 32°C in BAM broth.*

Test compound	Sporostatic MIC[a] (mM)	Sporicidal MIC (mM)
Benzaldehyde	0.60	625
Acetaldehyde	9.78	>2500[b]
Cinnamaldehyde	9.78	>2500
Piperonal	0.60	>2500
Isobutyraldehyde	0.60	39
Phenylacetaldehyde	0.60	>2500
α-Amylcinnamaldehyde	0.60	2500
Pyruvaldehyde	2.44	156
Vanillin	0.60	1.25
Phenylglyoxal	0.60	>2500
Heptaldehyde	0.60	>2500
Valeraldehyde	2.44	156

[a] Minimal inhibitory concentration.

[b] Indicates that none of concentrations tested inhibited spore germination.

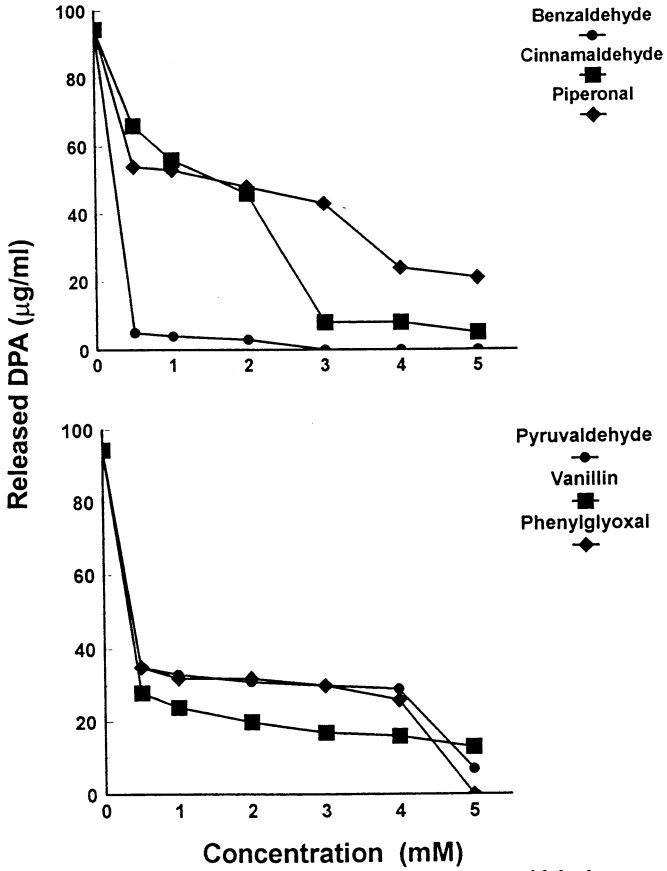


Figure 1. *The effect of aromatic and aliphatic aldehydes on dipicolinic acid (DPA) release from* C. botulinum *spores in BAM broth after 9 h anaerobic incubation at 32°C.*

Antigerminative properties were retained when the aldehydes were tested in commercially prepared beef and chicken broths. Four millimolars benzaldehyde, cinnamaldehyde, piperonal, pyruvaldehyde, vanillin, and phenylglyoxal delayed germination for 4 d at 25°C. However, 5.0 mM cinnamaldehyde, piperonal, and phenylglyoxal were required to inhibit germination for 8 d (Table 4).

MICs were higher than those observed for spores when the compounds were tested against *C. botulinum* cells at various pH values in BAM agar (Table 5). At pH 7.0, cinnamaldehyde, phenylglyoxal, and pyruvaldehyde were most active against *C. botulinum* cells; 25 mM was required. However, the inhibitory activity of isobutyraldehyde, α-amylcinnamaldehyde, and heptaldehyde were lost at pH values greater than 6.0, while the inhibitory properties of phenylacetaldehyde and pyruvaldehyde against *C. botulinum* cells remained constant at all pH values tested.

Five millimolars benzaldehyde, cinnamaldehyde, piperonal, pyruvaldehyde, or phenylglyoxal delayed 48 h neurotoxigenesis in BAM broth at 32°C (Table 6), while 25 mM was required for vanillin. Control cultures incubated under the same conditions were toxigenic at 48 h; botulism symptoms were observed within 2 h of injection.

DISCUSSION

Although several sporeforming bacteria are recognized as foodborne pathogens (*3*), few compounds are approved that can inhibit in foods germination or vegetative growth of these organisms (*28*). Therefore, aromatic and aliphatic aldehydes were assessed for antibotulinal activity. Of 12 compounds tested, 6 (pyruvaldehyde, phenylacetaldehyde, heptaldehyde, α-amylcinnamaldehyde, isobutyraldehyde and valeraldehyde) are Food and Drug Administration approved synthetic flavors, and 5 (piperonal, cinnamaldehyde, benzaldehyde, vanillin and acetaldehyde) were generally regarded as safe (GRAS) synthetic flavoring agents (21 CFR 172.515, 1992). Phenylglyoxal, however, is not an approved food additive but has been reported an effective inhibitor of anaerobes (*16*). The aldehydes tested in this study varied in their effectiveness against spores and cells of *C. botulinum*. Aromatic aldehydes, with few exception, had the lowest MICs. Of the eight aldehydes strongly inhibitory toward spore germination in BAM broth, 6 were aromatic, while aliphatics were less active. Phenylacetaldehyde, an aromatic, and pyruvaldehyde, an aliphatic, were the most active against cells of *C. botulinum*.

The activity of aldehydes against spores was further

TABLE 3. *Effect of selected aldehydes (100 mM) on thermal resistance of* C. botulinum *spores in BAM broth.*

Test compound	Population density (log_{10} CFU/ml) after thermal treatment (min) at 80°C					
	T_{nh}[a]	T_0[b]	T_{15}	T_{10}	T_{15}	T_{20}
Control[c]	6.92 ± 0.02[d]	6.92 ± 0.00	6.92 ± 0.00	6.92 ± 0.05	6.92 ± 0.00	6.92 ± 0.00
Benzaldehyde	6.91 ± 0.02	6.82 ± 0.02	5.91 ± 0.01	5.88 ± 0.01	5.57 ± 0.03	5.42 ± 0.02
Cinnamaldehyde	6.92 ± 0.00	6.85 ± 0.01	5.88 ± 0.01	4.91 ± 0.01	2.66 ± 0.01	2.28 ± 0.02
Piperonal	6.92 ± 0.01	6.86 ± 0.01	6.04 ± 0.02	5.88 ± 0.01	5.16 ± 0.01	2.96 ± 0.01
Pyruvaldehyde	6.91 ± 0.01	6.85 ± 0.00	6.05 ± 0.02	5.35 ± 0.00	2.26 ± 0.01	1.96 ± 0.01
Vanillin	6.90 ± 0.00	5.87 ± 0.03	5.96 ± 0.00	5.59 ± 0.00	4.94 ± 0.00	2.23 ± 0.02
Phenylglyoxal	6.91 ± 0.01	3.91 ± 0.00	3.87 ± 0.00	3.03 ± 0.00	2.05 ± 0.01	1.35 ± 0.03

[a] Nonheat-treated controls (incubated 50 min at 25°C).
[b] Population density at 80°C equilibration.
[c] Unsupplemented heat-treated control.
[d] Log_{10} mean ± standard deviation of the log_{10} CFU/ml counts of five replicate samples.

TABLE 4. *MIC required to prevent germination of* C. botulinum *spores in commercially prepared broths incubated for up to 8 d at 25°C.*

Aldehyde	Commercial broth	MIC[a] (mM) at incubation time (d)			
		2	4	6	8
Benzaldehyde	chicken	2.0	4.0	>5.0[b]	>5.0
	beef	2.0	4.0	>5.0	>5.0
Cinnamaldehyde	chicken	2.0	4.0	5.0	5.0
	beef	2.0	4.0	4.0	5.0
Piperonal	chicken	2.0	4.0	5.0	5.0
	beef	2.0	4.0	4.0	5.0
Pyruvaldehyde	chicken	2.0	4.0	5.0	>5.0
	beef	2.0	4.0	5.0	>5.0
Vanillin	chicken	2.0	4.0	>5.0	>5.0
	beef	2.0	4.0	>5.0	>5.0
Phenylglyoxal	chicken	2.0	4.0	5.0	5.0
	beef	2.0	4.0	4.0	4.0

[a] Minimal inhibitory concentration. Each compound was tested at 2.0, 3.0, 4.0, and 5.0 mM concentrations.
[b] Indicates that none of the concentrations tested delayed germination at the incubation time indicated. Growth was assessed by comparing test cultures with unsupplemented (inoculated and uninoculated) commercial broth controls.

TABLE 5. *Inhibitory activity of aldehydes in BAM agar against* C. botulinum *vegetative cells at 32°C.*

Test compound	48 h MIC[a] (mM) at pH value		
	6.0	7.0	8.0
Benzaldehyde	125	125	500
Acetaldehyde	125	125	500
Cinnamaldehyde	25	25	125
Piperonal	25	125	125
Isobutyraldehyde	125	>500[b]	>500
Phenylacetaldehyde	25	25	25
α-Amylcinnamaldehyde	500	>500	>500
Pyruvaldehyde	25	25	25
Vanillin	25	25	125
Phenylglyoxal	125	125	25
Heptaldehyde	25	>500	>500
Valeraldehyde	25	500	>500

[a] Minimal inhibitory concentration.
[b] No inhibition observed at the highest concentration tested. Unsupplemented controls were included for each of the pH values tested.

TABLE 6. *The effect of aldehydes on 48 h* C. botulinum *neurotoxigensis in BAM broth at 32°C.*

Test compound	MIC[a] (mM)
Benzaldehyde	5.0
Cinnamaldehyde	5.0
Piperonal	5.0
Pyruvaldehyde	5.0
Vanillin	25.0
Phenylglyoxal	5.0

[a] Minimal inhibitory concentration. Controls (no compound) were toxin positive at 48 h.

characterized by testing their effect on DPA release during germination. Several aldehydes were found to inhibit DPA release. The most active inhibitor was benzaldehyde, the simplest aromatic aldehyde tested, while pyruvaldehyde, an aliphatic, was the least effective. DPA (2,6-pyridinedicarboxylic acid) is a unique structural component that has been reported to induce germination of some sporeforming bacteria (*10,18*) and is essential to the development of bacterial spore resistance (*11,18*). DPA release occurs during the initial germination phase and marks conversion of dormant spores to vegetatively defined and sensitive structures. Bacterial spore germination is closely related to changes in the macromolecular configuration of structural components embedded in the spore coat (*9,10,13,18*). The conversion of a dormant spore to a germinated one is characterized by concurrent changes in permeability, access to a particular site, the unmasking of an enzyme or control site on an enzyme, and loss of a spore component (i.e., dipicolinic acid). As such, inhibition of DPA release suggests that antigerminative activity of the aldehydes tested may be attributed to their effect on spore coat components that stimulate events terminating the cryptobiotic state of *C. botulinum*.

Both aromatic and aliphatic aldehydes effectively reduced the thermal resistance of *C. botulinum* spores at 80°C; phenylglyoxal and pyruvaldehyde were most active. Since the temperature employed is usually innocuous against *C. botulinum* spores, any reduction in population density may be

construed as a marked change in spore resistance. The resistance of bacterial spores and bacterial cell adaptation under adverse environmental conditions is one parameter to define thermal processing requirements (*26,27*). Therefore, chemical additives, packaging, and other manufacturing technologies that reduce processing requirements and/or the resistance of bacterial cells and spores are desirable. As such, the reactive properties of aldehydes may be exploited in packaging/processing technologies to ensure the quality and stability of heat-sensitive products. Reduced thermal resistance of *C. botulinum* spores may be attributed to the binding of aldehydes to spore coat components, deleterious effects of free radicals formed during heat treatment, or heat-induced spore protoplast incorporation of aldehydes (*10,11,18,25*).

Since efficacy in application conditions is often different from those observed in model systems, antibotulinal activity was assessed in commercially prepared chicken and beef broths. Five millimolars cinnamaldehyde, piperonal and phenylglyoxal prevented germination for 8 d. However, vanillin, pyruvaldehyde, and benzaldehyde were less effective. Retention of sporostasis in commercial canned broths by aldehydes demonstrates their presumptive efficacy as antimicrobial agents in food products.

C. botulinum neurotoxin is the most potent known and is of primary concern in a variety of food products. All aldehydes tested for their activities against toxigenesis were effective as determined by bioassay. Vanillin, an aromatic aldehyde, was the least active of the compounds tested. The observed inhibition of neurotoxigenesis may be attributed, initially, to the antigerminative properties of aldehydes. During extended incubation, inhibition may be the result of reduced metabolic activities, or altered nutrient uptake and utilization by vegetative cells.

Aldehyde reactivity is dependent upon carbon chain length, functional groups, and physical and chemical nature of the environment (*21*). A number of structural trends were observed when the compounds were ranked according to their antibotulinal activities. The sporostatic MIC of benzaldehyde, phenylglyoxal, and vanillin was independent of carbonyl number and functional groups. Both sporicidal and antivegetative cell activities of benzaldehyde were enhanced by hydroxal and methyl group (i.e., vanillin) addition. In general, 5-9 carbon aliphatic aldehydes were most active against *C. botulinum* spores and cells. Similar observations have been reported for the antibotulinal activity of saturated aliphatic acids and butyrate esters (*8*). Attachment of acetaldehyde to benzaldehyde (i. e., phenylacetaldehyde) reduced the sporostatic MIC of acetaldehyde, antagonized the sporicidal MIC of benzaldehyde, while the vegetative MIC was unchanged. The addition of a 5-carbon side chain to the α-position of cinnamaldehyde (i.e., α-amylcinnamaldehyde) enhanced its sporostatic MIC and reduced its inhibitory activity against *C. botulinum* cells. Although antibotulinal activity was dependent upon aromaticity, chain length, and added functional groups, additional analyses with a greater number and variety of compounds are needed to confirm these observations.

Since most of the compounds tested are approved food additives, and in some instances generally regarded as safe (GRAS), their potential incorporation as antimicrobial agents is greatly enhanced. While sensory objections may limit use of these compounds for their sporocidal potential, antigerminative activity can be readily attained at acceptable levels. Furthermore, aldehydes that reduced *C. botulinum* thermal resistance demonstrate potential to reduce thermal processing requirements in commercially sterile or minimally processed refrigerated foods. These and other applications of aldehydes as antimicrobial food additives are currently under investigation.

ACKNOWLEDGMENTS

The technical assistance of Benne Marmer, B. Shawn Eblen, and Jeffrey E. Call is greatly appreciated.

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